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Feature

New fears over bee declines

Around the world, losses of bee colonies and wild pollinators continue. Emerging explanations are complex and call for more research, but the case against systemic pesticides is gaining strength. Michael Gross investigates.

Next year will mark the fiftieth anniversary of the publication of Rachel Carson's book *Silent Spring*, which alerted the world to the dangers that pesticides pose to the environment and contributed to the ban of DDT. In its wake, the growing awareness of environmental problems also led to the agrochemical industry looking for less harmful alternatives.

A new generation of chemicals, the so-called systemic pesticides introduced in the early 1990s, promised to address pests more specifically. The idea behind the new approach appeared elegant and convincing. Treating the seeds with a substance that would be incorporated into all plant cells (hence 'systemic') and kill insects that nibble on them, rather than blanketing the fields with toxins, one would be able to get the substance specifically to where it was needed and avoid collateral damage among desirable insects such as honey bees and wild pollinators. However, beekeepers and environmentalists are ever more vociferous in demanding a ban of the most widely used group of systemic pesticides, the neonicotinoids. What went wrong?

Throughout the current wave of honey bee losses, termed 'colony collapse disorder' (CCD) in the US since the catastrophic losses in 2006/7, neonicotinoids have been discussed as a possible culprit, simply based on the observation that they had just come into widespread use. An early ban was put into place in France in 1999, but the results remained inconclusive, as the situation of the bees didn't improve significantly after the ban. In Germany, a regional die-off of bee colonies in the spring of 2008 was found to be connected to inappropriate handling of the neonicotinoid clothianidin, used in products like Poncho Pro. While the producer, Bayer Crop Science, insisted that the product is safe when handled according to the instructions, it quickly agreed to pay a total of two million euros in compensation to the 700 beekeepers affected by the problem (see Curr. Biol. *18*, R684).

Like nicotine, neonicotinoids mimic the neurotransmitter acetylcholine (ACh), but they aren't recognised by acetylcholinesterase, the enzyme that normally inactivates ACh. Thus, false neural signals may accumulate over time. Even at levels that are far from fatal, this unwarranted neuronal excitation may confuse bees to an extent that they don't find back to their hive, which would explain the CCD symptom of deserted, but otherwise healthy-looking hives. Alternatively, the pesticides may also have unexpected cumulative effects in combination with pathogens like the fungus *Nosema*.

A research paper from Cédric Alaux and co-workers at Avignon, France, found the first clear evidence for such a synergy between the fungus infection and the pesticide (Environ. Microbiol. *12*, 774). The researchers observed significantly higher mortality in bees experimentally exposed to both *Nosema* and a concentration of imidacloprid corresponding to levels found in the environment than in bees exposed to only one of these factors. One possible mechanism behind this phenomenon is the energy requirement of the fungus, which puts significant energy stress on the host. By consuming more of the contaminated food than they normally would, bees may be pushed over the limit of lethal exposure.

Further evidence against neonicotinoids comes from the Netherlands, where the toxicologist Henk Tennekes from Experimental Toxicology Services Nederland at Zutphen has studied the distribution of neonicotinoids in the environment

Worries: Researchers are beginning to piece together some of the factors behind global honey bee population falls. (Photo: Andrew Moore/British Beekeeping Association.)

Crucial: Bumblebees have a vital role in pollination but are also in decline. (Photo: Michael Gross.)

and their possible effects on pollinators and on the animals further up the food chain. In September 2010, Tennekes summarised his findings, which are also published in several research papers, in a book called *The Systemic Insecticides: A Disaster in the Making*, leaving absolutely no room for doubt regarding his conclusions. Tennekes argues that the systemic poison not only affects insects eating the plants, but also those pollinating them.

Regarding the damage to non-target insects, Tennekes argues that "there may not be a safe level of exposure". The ACh receptors, he says, "play roles in many cognitive processes and neonicotinoids account for worker bees neglecting to provide food for eggs and larvae, and for a breakdown of the bees' navigational abilities. Very small quantities of neonicotinoid insecticides are sufficient to cause collapse of bee colonies in the long run."

An additional problem, according to Tennekes, is that the pesticides are also used as soil treatment and leach from the soil into the ground water, where they hit non-target insects and the birds that feed on them. The use of the neonicotinoid imidacloprid in the Netherlands increased ten-fold from 1995 to 2004, when over 6,000 kg of the chemical was applied to over 40,000 hectares. Since 2004, the Dutch Water Boards have reported significant contaminations of ground water with this neonicotinoid. "The excessive imidacloprid levels noted in surface water of western Dutch provinces with intensive agriculture have already been associated with insect decline and a dramatic decline of common grassland birds," Tennekes writes in his book.

In the UK, the invertebrate conservation charity Buglife commissioned a report in 2009, which concludes that the approval process for neonicotinoids and for the unrelated substance fipronil, which also acts as a systemic pesticide, was inadequate. The report argues that the approval had not taken into account the possible consequences of sublethal exposure to systemic substances in non-target insects such as bees. In addition, the report notes that "there were also a number of exposure routes that had not been properly investigated, such as exposure from dust formed during the sowing of dressed seeds."

Confirmation for these concerns comes from the US, where leaked documents suggest that the US government's bee lab in Beltsville, Maryland has results showing that imidacloprid increases the susceptibility of bees to infectious diseases, even at the smallest doses investigated. These results remained unpublished for two years, but they are in essential agreement with the published work from Alaux and colleagues at Avignon.

Based on the Buglife report and the news of the US government lab, a group of 56 MPs in the UK supported an Early Day Motion filed last month, calling on the government "to act urgently to suspend all existing approvals for products containing neonicotinoids and fipronil pending more exhaustive tests and the development of international methodologies for properly assessing the long-term effects of systemic pesticides on invertebrate populations." However, the government declined to change its policy. A Department for Environment, Food and Rural Affairs spokesman said: "The UK has a robust system for assessing risks from pesticides and all the evidence shows neonicotinoids do not pose an unacceptable risk when products are used correctly. However, we will not hesitate to act if presented with any new evidence."

In the same week, it emerged that the British Beekeepers Association (BBKA) had received £175,000 from pesticide manufacturers including Bayer, BASF, and Syngenta, in a deal allowing the companies to use the BBKA logo and claim that their substances are 'bee-friendly'. Many members of the association had not been aware of the deal struck by the executive committee and responded furiously when the issue was raised in an open letter from environmentalists. The BBKA promptly ended its agreement with the companies, a decision endorsed by the annual delegates meeting with a large majority. However, the decisions voted through by the delegates did not rule out similar deals with companies in the future.

While honey bees are extremely important for food production around the world, wild pollinator species are also very important for the plants that feed us. Most prominent among them are the bumblebees, which are in fact more efficient pollinators than honey bees. Championing the bumblebee as "environmental mascot for Britain" at the most recent annual Earthwatch debate at London's Royal Geographical Society, the zoologist and TV presenter George McGavin pointed out that with 25 native species of bumblebee, the British Isles host 10% of the global bumblebee fauna. Three of these species have become extinct already, and around half of the remainder are believed to be in decline due to habitat loss. However, some bumblebee species are thriving on the diverse offerings of British gardens, and one species, *Bombus hypnorum* (tree bumblebee), has steadily expanded its range in Europe and has colonised British gardens since 2000.

A first systematic investigation of bumblebee species range in North America comparing current and historical distribution found that, of the eight species investigated, four were declining and now restricted to a significantly smaller range (Proc. Natl. Acad. Sci. USA *108*, 662). Looking for possible causes, the researchers found that the declining species had a significantly higher prevalence of infection with *Nosema*. They also discovered that the declining populations had reduced genetic diversity compared with the stable ones. Establishing causal links between these observations, however, will require further research, the authors say.

The authors also observe that the species affected by decline in North America have previously had a wide climatic range. By contrast, studies in Europe have found that species with a narrow climatic range are most at risk. This contrast suggests that different causes and mechanisms may be behind the decline on both continents.

The simultaneous threats to both the domesticated honey bees and the wild pollinators are bound to have repercussions throughout the natural environment and are also putting agricultural production and food supplies at risk. George McGavin commented: “The global threat to bees is a greater threat to our survival than global warming. This is a total ecological disaster we can avoid.” Considering the scale of the industries affected, government spending on bee health has remained minuscule. McGavin calls the £1 million support that bee researchers get from the UK government “laughable”. The EU has so far been inactive, but in January the European Commission acknowledged the importance of the problem and announced the installation of a European reference laboratory for bees’ health to be based in France.

Tennekes concludes his analysis of the impact of neonicotinoids on wildlife in the Netherlands: “Ground and surface water contamination with persistent insecticides that cause irreversible and cumulative damage to aquatic and terrestrial (non-target) insects must lead to an environmental catastrophe. The data presented here show that it is actually taking place before our eyes, and that it must be stopped.”

More research and political action is required to ensure that we don’t, after all, experience what Rachel Carson anticipated 50 years ago: a silent spring.

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Quick guide

Neurospora

Eric U. Selker

What is* Neurospora?** *Neurospora* is the genus of a group of filamentous fungi but the word is most often used as a nickname for the best studied species, ***N. crassa, which has served as a model eukaryotic organism for nearly a century. The name *Neurospora* apparently came from the nerve-like stripes found on its sexual spores (‘ascospores’; Figure 1). *Neurospora* is easily recognizable by its orange aerial asexual spores (‘conidia’).

What is its life style? The haploid vegetative filaments (‘hyphae’), which look somewhat like axons (Figure 2), weave together to form a mat (‘mycelium’). *Neurospora* grows at a prodigious rate — the mycelium advances at ~4 mm per hour in a reasonably warm environment if given some sugar, simple nutrients, and one vitamin (biotin). ***N. crassa*** is ‘heterothallic’ meaning that it has different subtypes (‘mating types’) that must find each other to enter the sexual phase of the life cycle. About 10 days later, its fruiting bodies (‘perithecia’) shoot the ascospores towards light. Germination of ascospores requires heat (for example, 65°C for an hour), which kills other cells in the neighborhood and accounts for reports of *Neurospora* in French bakeries in the 1800s and for the presence of *Neurospora* in burned sugar cane fields and burned forests in modern times.

What was* Neurospora *first known for? Research in the 1920s and 1930s revealed ***N. crassa*** to be a convenient and powerful genetic system; indeed it became a textbook example of first-division and second-division segregation, with easily demonstrable crossing over at the four-strand stage, and provided the first proof of gene conversion. The fact that it could be easily grown on defined media led to its adoption for the Nobel-prize winning ‘one gene–one enzyme’ work of Beadle and Tatum in the 1940s, which demonstrated that genes

Figure 1. A dissected perithecium of *N. crassa* with octets of ascospores (stripes not visible at this magnification) showing segregation of a color marker (courtesy of N. Raju).